

## CLAIMS

What is claimed is:

1. A C3A clonal cell line derived from a parental C3A cell line, wherein said clonal cell line has a doubling time in serum-free medium significantly less than the doubling time of said parental line in said serum-free medium.
2. The clonal cell line of claim 1, wherein the doubling time in serum-free medium of said clonal cell line is less than about 70% of the doubling time in serum-free medium of said parental C3A cell line.
3. The clonal cell line of claim 1, wherein said doubling time in serum-free medium of said clonal cell line is in the range of less than about 50% to less than about 70% of the doubling time in serum-free medium of said parental C3A cell line.
4. The cell line of claim 1, wherein cells of said cell line cultured in serum-free medium express a single or any combination of a plurality of harvestable polypeptides.
5. The cell line of claim 4, wherein said cells express alpha fetal protein (AFP).
6. The cell line of claim 4, wherein said cells express human albumin.
7. The cell line of claim 4, wherein said cells express  $\alpha$ -1-antichymotrypsin.
8. The cell line of claim 4, wherein said cells express  $\alpha$ -1-antitrypsin.
9. The cell line of claim 4, wherein said cells express antithrombin III.
10. The cell line of claim 4, wherein said cells express complement C3.

1 11. The cell line of claim 4, wherein said cells express Factor V.

1 12. The cell line of claim 4, wherein said cells express transferrin.

1 13. The cell line of claim 4, wherein said cells express a single or any combination of a  
2 plurality of harvestable polypeptides selected from the group consisting of: alpha fetal  
3 protein (AFP), human albumin,  $\alpha$ -1-antichymotrypsin,  $\alpha$ -1-antitrypsin, antithrombin III,  
4 complement C3, Factor V and transferrin.

1 14. The cell line of claim 1, said cell line having an ATTC accession No. of CRL-12461.

1 15. A method of producing a single or any combination of a plurality of harvestable  
2 polypeptides, comprising:

- 3 a) culturing cells of the cell line of claim 1 in serum-free medium,
- 4 b) expressing said polypeptide/s from said cells; and
- 5 c) recovering said polypeptide/s from said culture to produce a harvestable polypep-  
6 tide.

1 16. A method of producing the cell line of claim 1, comprising:

- 2 a) sequentially culturing cells of a parental C3A cell line in a series of medium  
3 having incrementally decreasing concentration of serum, the final medium in said  
4 series being serum free,
- 5 b) generating a clonal cell colony of said cells from said final medium in said series  
6 of a) in serum-free medium; and
- 7 c) propagating said colony in serum-free medium to produce a serum-free cell  
8 line.

1 17. The method of claim 16, wherein one of said series of medium having incrementally  
2 decreasing concentration of serum in said sequential cultures series has a ratio of serum  
3 containing and serum-free medium of about 50:50.

1 18. The method of claim 16, wherein one of said series of medium having  
2 incrementally decreasing concentration of serum in said sequential cultures series has a  
3 ratio of serum containing and serum-free medium of about 25:75.

1 19. The method of claim 16, wherein the serum-free medium is JRH Bioscience ExCell  
2 620 supplemented with 2mM L-glutamine.

1 20. A bio-artificial liver device comprising an apparatus containing cells of the cell line  
2 of claim 1, wherein said cells are cultured in serum-free medium on a surface in said  
3 device in an amount and having liver specific biological activity at a level sufficient to  
4 sustain a subject having a liver disorder or compromised liver function.

1 21. A method of using cells of the cell line of claim 1 in a bio-artificial liver device,  
2 comprising providing said cells to a surface in said device and culturing said cells in said  
3 device in serum-free medium.

1 22. A method of treating a subject having compromised liver function, comprising:  
2 a) providing cells of the cell line of claim 1 to a surface in a bio-artificial liver  
3 device, wherein said cells are provided in an amount and having liver specific  
4 biological activity at a level sufficient to sustain said subject having said compro-  
5 mised liver function,  
6 b) culturing said cells in said device in serum-free medium; and  
7 c) passaging blood from said subject to contact said cells, wherein said passaging  
8 results in removal of blood-borne molecules entering said device and release of  
9 molecules from said cells into blood exiting said device.

1 23. The method of claim 22, wherein said device is outside said subject.

1 24. The method of claim 22, wherein said device is inside said subject.

1 25. The method of claim 22, wherein said subject is a human.

1 26. A method of producing protein comprising:

- 2 a) culturing cells of any of claims 1 to 14 in serum-free medium to express a single  
3 or any combination of a plurality of harvestable polypeptides; and  
4 b) recovering said polypeptide/s to produce protein.

1 27. A method of screening compounds for metabolic activity comprising:

- 2 a) providing a compound to cells of the cell line of claim 1, wherein said cells are  
3 cultured in serum-free medium; and  
4 b) analyzing said cells for the presence of metabolites of said compound to screen  
5 for metabolic activity.

1 28. A method of studying enteric disease comprising:

- 2 a) providing a bacterial organism to cells of the cell line of claim 1, wherein said  
3 cells are cultured in serum-free medium; and  
4 b) employing said cells of a) for experimental use to study enteric disease.